THE NATURAL-SELECTION THEORY OF ANTIBODY FORMATION

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An immense amount of experimental data related to the problem of antibody formation has accumulated. Theories offering a basic interpretation of these observations have, in contrast, been few. The theory formulated in the present paper, though highly speculative, attempts to provide a framework for the interpretation of the main features of antibody appearance in response to the injection of antigen into an animal.

Two views concerning the mechanism of antibody formation are at present most widely favored. One is the "antigen-template" theory, developed by Breinl,[1] Haurowitz,[1,2] Mudd,[3] Alexander,[4] and Pauling.[5] This theory assumes that antibodies can be produced only by cells in which the antigen is present. The specific affinity of an antibody molecule toward the antigen is due to a complementarity in structure derived from the folding of part of the polypeptide chain of a globulin molecule in direct contact with a determinant or haptenic region of the antigen. The antigen thus serves as a template in the final stage of formation of a globulin molecule.

The other view tries to establish a similarity between antibody formation and adaptive enzyme formation and allows for the continued production of antibody after the antigen has disappeared from the body. This is the "modified-enzyme" theory, formulated by Burnet[6,7] and Fenner.[7] They propose that the introduction of an antigen into cells, containing enzymes directed toward the disposal of effete cells and cellular debris from the organism itself, induces the formation of "enzymic units" adapted toward the destruction of the antigen. A renewed contact with the antigen stimulates the replication of these enzymic units. Circulating antibody molecules are partial replicas of the modified enzymic units, carrying specificity but lacking enzymic action.

The "natural-selection" theory, proposed in the present paper, may be stated as follows: The role of the antigen is neither that of a template nor that of an enzyme modifier. The antigen is solely a selective carrier of spontaneously circulating antibody to a system of cells which can reproduce this antibody. Globulin molecules are continuously being synthesized in an enormous variety of different configurations. Among the population of circulating globulin molecules there will, spontaneously, be fractions possessing affinity toward any antigen to which the animal can respond. These are the so-called "natural" antibodies. The introduction of an antigen into the blood or into the lymph leads to the selective attachment to the antigen surface of those globulin molecules which happen to have a complementary configuration. The antigen carrying these molecules may then be engulfed by a phagocytic cell. When the globulin molecules thus brought into a cell have been dissociated from the surface of the antigen, the antigen has accomplished its role and can be eliminated.

The introduction of the selected globulin molecules into a cell or the transfer of these molecules into another cell is the signal for the synthesis or reproduction of

molecules identical to those introduced, i.e., of specific antibodies. The release of these antibody molecules into the circulation will shift the composition of the population of circulating globulin molecules. Antigen, secondarily introduced into the circulation, now meets a larger concentration of specific molecules and carries a larger quantity of these, selected for the better-fitting ones, to the antibody-producing apparatus, which already contains many cells engaged in the synthesis of molecules of these types of specificity. This leads to the more rapid reproduction of an improved assortment of antibody molecules, followed by a further directional shift in the circulating globulin population. The reproduction need not be highly faithful; copying mistakes will be harmless, and may occasionally produce an improved fit. When, in the absence of further antigen stimuli, no more pressure is exerted, the population will slowly revert toward a normal condition of equilibrium. The normal equilibrium may be upheld by a continuous reproduction of samples of the population of molecules circulating at any given moment. Somewhere, however, either in the beginning of the life of an animal or continuously, a spontaneous production of random specificities must take place. The spontaneously produced globulin molecules may be formed only in small numbers. Those among them that will attach themselves to structures in the body of the animal itself will be removed and will therefore not be available for reproduction. The absence of globulin molecules carrying these specificities will prevent a response to antigens of these specific types.

Discussion.—Burnet and Fenner[7] list a number of essential immunological observations which are not satisfactorily accounted for by the antigen-template theory.

1. The booster effect. A secondary stimulus with the same antigen provokes a more active production of antibody than does a primary stimulus. According to the natural-selection theory, this would be due to the fact that antigen injected secondarily encounters a larger concentration of specific antibodies in the circulation than were present at the time for the primary injection. More antibody molecules are therefore brought to the globulin-reproducing cells.

2. The change in character of the antibody produced in response to repeated inoculations of the same antigen. The main changes observed are from "low-grade" antibody of low combining capacity, produced in the beginning of an immunization course, toward "more avid" antibody of high combining power, produced later.[7–10] Besides this improvement in quality, an increase has been observed in the range of cross-reactions with related antigens. The present theory explains this development by natural selection. At the time of the primary stimulus the antigen injected finds only few globulin molecules in the circulation, showing various degrees of affinity toward the antigen surface patterns. At the time of a later stimulus in the course of immunization, when these molecules have been replicated in large numbers, the antigen will find a larger concentration of globulin molecules fitting all its surface patterns and will preferentially carry those which show the highest combining capacity to the globulin-reproducing cells.

3. The apparent exponential rise in circulating antibody during the first period of production. On the present theory this may be due to an autocatalytic replication of the specific globulin molecules and to a multiplication of the cells.

4. The continued production of antibody for long periods. The antigen-template theory could deal satisfactorily with this point if it could be shown that

sufficient antigen remains present during the entire period of antibody production.[11] On the other hand, this theory would have to be abandoned if it could be shown that antibody production continues after the antigen has been eliminated from the body.[12] The latter view is held by Burnet and Fenner, for the following reasons: Since circulating antibody has a rapid turnover, it must be continuously synthesized for long periods, and not only by the cells originally stimulated. The reticulo-endothelial cells and the plasma cells which are believed to be engaged in antibody production are short-lived cells. The repeated transfer of the original antigen from a disintegrating cell to another appropriate cell seems unlikely. According to the natural-selection theory, the continued antibody production after elimination of the antigen is natural, because the antigen will have completed its role when it has carried the antibody molecules to the reproducing apparatus.

5. To this list we would add the observation that the surface of particulate antigens seems to play a dominant part in determining the specificity of the antibody molecules produced. Avery and Neil[13] found that the antibody produced against suspensions of pneumococci is mainly directed against the surface presented by these bacteria. White[14] who studied the antibody produced as a response to injections of *Salmonella* cells, concluded that "it would seem that the antiserum of the bacillus is overwhelmingly the antiserum of its surface rather than of its substance." Henle *et al.*[15] studying spermatozoa as antigens, and Morgan[16] who investigated the antigens of *Shigella,* arrived at similar conclusions. This predominance of the antigen surface is in harmony with the natural-selection theory of antibody formation because the globulin molecules that are eligible for preferential reproduction are those that can attach themselves to the surface presented by the antigen before it is removed from the circulation. It would seem, however, to involve the antigen-template theory in difficulties. From the well-known experiments of Topley[17] it can be calculated that a rabbit, after two intravenous injections of formalin-killed *Salmonella* cells, synthesized about 100,000 antibody molecules per second per bacterium that had been injected, and continued this rate of production for a period of many weeks. If we imagine that the bacteria serving as antigen templates were not broken down but resided *in toto* each in an antibody producing cell, this would entail impossible quantitative implications. If, however, the bacteria were broken down into many fragments, how would a cell in which one of these fragments served as antigen template be able to distinguish what part of the fragment had originally been at the surface of the intact bacterium?

It seems worthwhile to consider more closely a theory which seems to provide simple explanations of these important immunological phenomena. Except for specificity toward an antigen, no known properties distinguish normal serum globulin from antibody. In the absence of antigen no directional pressure is imposed upon globulin synthesis, and it seems reasonable to assume that a great variety of configurations, due, perhaps, to various amino acid sequences at the specific sites of the globulin molecules, may develop at random.

An immense body of experimental data testifies to the fact that normal sera from one animal species may contain antibodies against an enormous number of different bacteria, some of which are not known to be natural parasites to this species.[18] Though these natural antibodies are often supposed to have evolved as a result of previous exposure to antigen, this attitude seems merely to reflect the definition of

an antibody as a substance produced in response to an antigen, which, however, does not imply that antibodies are not produced in the absence of, and prior to, exposure to an antigen. Doerr,[19] summing up an extensive review of experimental data in this field, states: "We must accept that it has been definitely demonstrated that natural antibodies can develop without an antigenic stimulus, and that this spontaneous formation is by far the most frequent origin of natural antibodies against bacteria erythrocytes, toxins, and virus particles." The slight phagocytosis promoting activity found in normal sera has been ascribed to the presence of normal specific opsonins. These substances may be equated with natural antibodies. Their activity can be specifically inhibited by hapten. It has been observed[20] that the ability of a rabbit to produce antibodies against bovine serum albumin or against an artificially conjugated antigen is related to the rate at which the injected antigen is removed from the blood. In poorly responding animals the antigen remains in circulation much longer than in good responders. We interpret this in terms of the spontaneous presence in the circulation of less or more specificially fitting globulin, which determines both the rate of phagocytic removal and the amount of specific globulin that will be carried to the globulin-reproducing system.

The methods available for demonstrating the presence of specific antibody in serum rarely permit the detection of concentrations lower than 10^{12} or 10^{11} antibody molecules per milliliter. Even diphtheria antitoxin, by the extremely sensitive rabbit skin test, cannot be detected in dilutions containing less that 5×10^{10} antitoxin molecules per milliliter.[8] Most sensitive, probably, is the demonstration of antibodies against bacteriophage,[10,21] which can be measured quantitatively in sera from normal animals.[22]

Among the comparatively small number, perhaps a few thousand, of antigen-antibody systems investigated, cross-reactions are by no means rare, suggesting that the number of specific configurations which a globulin molecule can exhibit is large but limited. Since normal mammalian serum contains more than 10^{17} globulin molecules per milliliter, these may include a million 10^{11} fractions of different specificity. This would seem an amply sufficient number.

In what type of cells does the postulated replication of globulin molecules take place? The lymphoid tissue contains cells which are capable of producing globulin and antibody.[23] This tissue is scattered throughout the body but is mainly concentrated in the bone marrow, the thymus, the spleen, the appendix, and various lymph nodes, totaling about 0.5 per cent of the body weight. The mesenchymal reticulum cells of the lymphoid tissue develop through a maturation series to one of three types of cells: phagocytic endothelial cells, lymphocytes, and plasma cells. The developmental and functional relationship between these closely associated cells is not clear. The phagocytic cells take up foreign particles from the blood and the lymph, and, since the blood is filtered through bone marrow and spleen, and the lymph through the lymph nodes, circulating phagocytes also come into close contact with the lymphoid tissue. Lymphocytes have a life-span of not more than a few days. It can be calculated that the daily output in the rabbit is of the order of 10^{10} of these cells. They circulate in the lymph and blood, and many seem to return to the germinal centers of the lymphoid tissue. The lymphocytes seem to produce some gamma globulin, but whether they take part in the production of antibody after an antigen stimulus is undecided. The plasma cells, on the other hand,

are definitely involved. In conditions of hyperglobulinemia and of hyperimmunization there is a large increase in plasma cells in the bone marrow and elsewhere. The injection of an antigen into the tissues of an animal leads to the production of antibody in the lymph node draining the injection site. During the days following the injection this lymph node exhibits marked mitotic activity. There is an increase in weight and a parallel increase in desoxyribonucleic acid (DNA). This is followed by a large increase in ribonucleic acid (RNA), which reaches a peak around the fifth day, coinciding with the period of greatest antibody increase. The RNA is mainly concentrated in the freely multiplying plasma cell precursors. The antibody content of the lymph leaving the lymph node may be a hundred times greater than that of the lymph entering the node, and most of this antibody leaving the node is inclosed in cells suspended in the lymph plasma, about 5 per cent of which may be plasma cells, the rest lymphocytes. After intravenous injections antibody-producing plasma cells can be recognized in the red pulp of the spleen.

The thymus, though rich in nucleic acid and lymphocytes, develops no plasma cells and does not show any immunological activity, Burnet and Fenner,[7] in spite of this, suggest that the lymphocytes may be responsible for maintenance of low levels of antibody long after the plasma cells have initiated the formation during the peak phase of the antibody response. Though they infer that, if this suggestion is true, the immunological function of the lymphocytes can hardly be their major one, it would be a different matter if the function included the maintenance of the circulating gamma globulin. In a rabbit the half-life of circulating globulin is about 5 days.[24] This means that the rabbit must daily synthesize about 10^{18} globulin molecules. For 10^{10} lymphocytes to accomplish this task, each would have to synthesize about a thousand globulin molecules per second.

If it were true that antibody production after an antigen stimulus is the preferential replication of selected globulin molecules, the production of normal globulin might be an unselective reproduction of the circulating globulin. This could be accomplished either by a mechanism by which a small fraction of circulating globulin could enter into lymphoid cells or by the transfer of synthesizing units from worn-out lymphocytes and plasma cells, returning to the lymphoid tissue, to young cells. In this way the animal would tend to preserve the spectrum of globulin specificities already present, and a drastic change could be accomplished only by a mechanism of preferential replication of a selected fraction of the circulating globulin. This would essentially reduce the production of both normal gamma globulin and antibody to one mechanism: selective and unselective reproduction of circulating globulin molecules.

Somewhere in the beginning, however, we have to postulate a spontaneous production of globulin molecules of a great variety of random specificities in order to start the process. Possibly a specialized lymphoid tissue, such as that of the thymus which is most active in embryonic and early independent life and decays soon after, is engaged in this function. If this small spontaneous production of globulin took place mainly in embryonic and early life, before the much larger body of cells later engaged in maintaining the composition of the circulating globulin by reproduction had started to function, the early removal of a specific fraction of molecules might lead to the permanent disappearance of this type of specificity. Such a mechanism could explain the absence in the blood of specific globulin against antigens of the

organism itself, since such globulin molecules, if spontaneously produced, would be removed by attachment to the auto-antigens and would no longer be available for reproduction. The absent specificities would include, besides auto-antibodies, natural antibody against antigens implanted in the animal during embryonic life. The absence from the circulation of such antibodies would, in turn, prevent response to a later antigenic stimulus of this type.[34]

This might occur also if a specific type of globulin molecules could be removed from an adult animal, and might be the explanation of the "immunological paralysis" described by Felton:[25, 26] the blocking of subsequent response by the administration of a large initial dose of pneumococcal polysaccharide. This hapten will induce the formation of antibody in mice only if the dose injected does not exceed a certain very small amount. Felton reported that only doses below 0.001 mg. of a certain preparation of such a polysaccharide were effective in engendering antibody formation in mice. Thus the injection of more than 10^{14} molecules of polysaccharide of molecular weight 4,000 inhibited the response. Perhaps a certain fraction of the haptenic particles, by association with a protein, are capable of antigenic stimulation but cannot act when the larger, purely haptenic fraction eliminates all the spontaneously present antibody molecules.

The crucial point of the natural-selection theory is the postulate that the introduction of antibody molecules into appropriate cells can be the signal for the production of more of their kind. This notion is unfamiliar. However, as nothing is known about the mechanism of antibody synthesis in a cell, it would seem a priori more reasonable to assume that an animal can translate a stimulus, introduced by protein molecules which it has itself at one time produced, into an increased synthesis of this same type of molecules than to suppose that an animal can utilize all sorts of foreign substances and can build them functionally and semipermanently into the most intimate parts of its globulin-synthesizing cells.

Any attempt to elaborate this notion into a picture of what may be the mechanism of a replication of specific protein must, at present, be highly speculative. It would seem profitable if, as the modified-enzyme theory tries to do, an analogy could be established on the cellular level between the induction of antibody synthesis in animals and the induction of adaptive enzyme synthesis in microörganisms, since both entail the increased formation of a specific protein. Recent discussions[27] of the nature of adaptive enzyme formation are, however, also still in a speculative stage.

Prior to induction, a small amount of the enzyme appears already to be spontaneously present in the adaptable cells. Induction may involve the replication either of the enzyme molecules or of the elements which form the enzyme.[28]

Since RNA seems to play a part in the organization of protein synthesis, it has been suggested that RNA acts as a template on which amino acids are assembled. RNA may thus determine the order of amino acid residues in the protein chain, and, reciprocally, as suggested by Caldwell and Hinshelwood,[29] a protein molecule may determine the order of the nucleotides in the synthesis of RNA. A reversible situation of this kind, as Gale[30] has pointed out, might account for his finding that labeled amino acids are incorporated into proteins of cells that have been prevented from synthesizing new protein by the lack of other essential amino acids. RNA does not seem to be synthesized unless amino acids are present, and optimal RNA synthesis occurs under conditions of optimal protein synthesis.[31] If these syntheses

were coupled, it would seem feasible that the introduction of a protein molecule into a cell could initiate a replication of its specific structure.

A mechanism of this sort would be needed for the present theory of antibody formation. If RNA is the template on which protein molecules are assembled, an antibody molecule introduced into the appropriate cell must be able either to initiate the synthesis of specific RNA or to combine with pre-existing RNA. The latter possibility would imply either that the cells already contain a large variety of RNA structures of various specificities—i.e., that a cell is already potentially capable of synthesizing a large variety of globulin molecules of different specificity, the desired type being favored by the introduction of a globulin molecule of this type—or that there are only a smaller variety of different RNA structures present in the cell, upon a related one of which the inducing antibody molecule can impose its specificity.

The present theory predicts that the amount of antibody produced in response to an antigen stimulus depends on the concentration of a type of circulating globulin which can attach itself specifically to the surface of this antigen. This could be tested in experiments of the following types.

The rate of antibody production after a primary stimulus should depend on the concentration of spontaneous antibody already present in the circulation. It is a common observation that the response to an antigen of an animal whose serum already contains a low level of specific antibody is greater than that of animals in which no antibody can be demonstrated.[32] It should be possible, therefore, to increase the rate of antibody production by increasing the concentration of antibody circulating at the time of the primary antigenic stimulus. By introducing antibody from an immunized donor animal into the blood of a recipient animal, prior to the injection of the antigen into the latter, enhanced antibody response to this antigen stimulus should be expected. The common finding in such an experiment is a depression of the response. However, experiments designed to test this point should take into account the fact that even when an animal of the same species is used as the donor of antibody, individual immunological differences residing in the globulin molecules may prevent the recipient animal from utilizing the globulin molecules supplied by the donor. Experiments would therefore have to be carried out between identical twins.[33] Also, when serum from a donor animal is used, the possibility should be considered that the antibody molecules may have been altered, during clotting, separation, and storage of the serum, from the form in which they existed in the circulating plasma.

Also, it should be possible to decrease the rate of antibody production by decreasing the concentration of antibody circulating at the time of the antigenic stimulus. Thus the injection of the corresponding hapten into the blood, prior to the antigen, should depress the antibody response. This may be the explanation of Felton's experiment[25] mentioned above.

The booster effect, i.e., the greater response to a secondary antigenic stimulus than to a primary one, should depend on the presence, at the time of the secondary stimulus, of antibody due to the primary stimulus. It has been observed that a booster response to diphtheria toxin does not occur in an animal after the effects, produced by the primary stimulus, have worn off and antitoxin is no longer demonstrable.

In cases in which cross-reactivity between two pairs of antigen-antibody systems is due to related surface patterns, a secondary stimulus with the second antigen

following a primary stimulus with the first antigen may lead to the increased production of antibody of the first type. The second antigen would carry many molecules, formed in response to the first antigen, to the reproducing cells. This is what is usually called the "anamnestic reaction."

Summary.—A theory of antibody formation is proposed which postulates the spontaneous presence, in the blood of an animal, of small numbers of antibody molecules against all antigens to which the animal can respond, and delegates to the antigen the sole role of carrying such specific globulin molecules from the circulation into cells in which these molecules can induce the production of more of their kind.

The theory offers an explanation for the presence in blood of a large pool of normal globulins, for the presence of natural antibodies, for the dominant part played by the surface of antigen particles in antibody induction, for the change in character of antibody during the course of immunization (by natural selection), for the exponential increase during part of antibody production, for a continued production of antibody in the absence of the antigen, for the booster phenomenon, for the absence of auto-antibodies, for immunological paralysis and haptenic inhibition, and for the anamnestic reaction.

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*THE INFLUENCE OF PLOIDY AND DIVISION STAGE ON THE ANOXIC PROTECTION OF SACCHAROMYCES CEREVISIAE AGAINST X-RAY INACTIVATION**

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It has been shown that for the lethal effect of X-rays on haploid yeast the dose is effectively halved for cells irradiated in the absence of oxygen.[1] The knowledge that in yeast the onset of budding is accompanied by a great decrease in sensitivity to the lethal action of X-rays[2] and alpha particles[3] raises the question of internal protection at this time, possibly of the same sort as that conferred by externally imposed anoxia. If this were the case, one would expect anoxia to provide little or no protection to cells in the process of budding. The additivity experiments to be reported here accordingly employed cells from actively growing cultures to insure the presence of adequate percentages of budding cells.

In addition to the standard haploid strain of *Saccharomyces cerevisiae* (SC-7), the parental diploid (SC-6) and a derived tetraploid (X-33)[4] were studied. All cultures were grown aerobically at 30° C. on yeast extract glucose agar, harvested for experiments after 12–18 hours of growth, and spread on Petri dishes containing this medium for irradiation and postirradiation aerobic incubation (3 days at 30° C.). Survival ratios were obtained from colony counts of irradiated plates by reference to unirradiated controls.

Detailed accounts have been given both of the radiation source used, a low-voltage X-ray tube (Machlett OEG-60) operated at 50 kv. and 25 ma.[4], and of the procedures employed in handling the yeast cells.[2] The chamber used to maintain a nitrogen atmosphere for anoxic irradiations is shown in Figure 1. Tank nitrogen